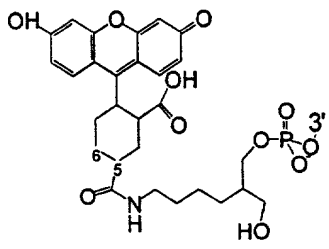
conditions during oligonucleotide attachment to the nanoparticles to gain high oligonucleotide surface coverages, oligonucleotide spacer segments to reduce electrosteric interactions, and coadsorbed diluent strands to reproducibly control the average number of hybridization events for each nanoparticle. It has also been shown that the nature of the tether (spacer) sequence influences the number of oligonucleotide strands loaded onto gold nanoparticles. This work has important implications regarding understanding interactions between oligonucleotides and nanoparticles, as well as optimizing the sensitivity of nanoparticle-oligonucleotide detection methods.

TABLE 7

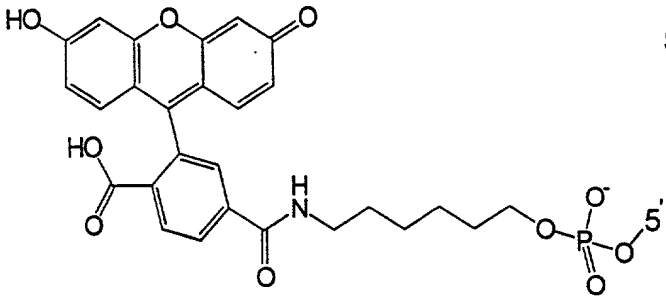
Single strand surface coverage and corresponding hybridized surface coverages for gold thin films and gold nanoparticles. Comparison between S12F and SA_{20}12F surface coverage and hybridization. Thiol modified oligonucleotides were attached to the gold from 3 μM aqueous solutions and aged in 0.1 M NaCl. All hybridization studies were performed in 0.3 M PBS, pH 7.

Oligonucleotide Pair	Surface Coverage ($pmol/cm^2$)	Hybridization Coverage ($pmol/cm^2$)	% Hybridization Efficiency
	Au nanoparticles		
S12F/12F'	34 ± 1	1.3 ± 0.2	~ 4%
SA_{20}12F/12F'	15 ± 4	6.6 ± 0.2	~ 44%
	Au thin films		
S12F/12F'	18 ± 3	6 ± 2	~ 33%



3' TAG-GAC-TTA-CGC-O(O)P(O)O-$(CH_2)_6$SH 5' = S12F'

SEQ ID NO:50



SEQ ID NO:54

TABLE 8

Effect of salt aging on surface coverage of $SA_{20}12F$ oligonucleotides to gold nanoparticles and hybridization to 12F'. All hybridization experiments were performed in 0.3 M PBS, pH 7.

Buffer conditions during adsorption of alkylthiol DNA	Surface Coverage (pmol/cm^2)	Hybridization Coverage (pmol/cm^2)	Hybridization Efficiency (%)
H_2O	7.9 ± 0.2	–[a]	–
0.1 M NaCl, 10 mM phosphate	15 ± 4	6.6 ± 0.2	~44
1.0 M NaCl, 10 mM phosphate	20 ± 2	6.5 ± 0.2	~33

[a] Reliable values for these experiments could not be obtained due to a small amount of particle aggregation which occurred after centrifugation.

TABLE 9

Effect of oligonucleotide spacer sequence on surface coverage and hybridization efficiency.

Oligonucleotide Pair	Surface Coverage (pmol/cm^2)	Hybridization Coverage (pmol/cm^2)	Hybridization Efficiency (%)
S3'A_{20}12F / 3'12F	24 ± 1	9 ± 2	~38
S3'T_{20}12F / 3'12F	35 ± 1	12 ± 1	~34

S3'A_{20}12F / S3'T_{20}12F = $HS(CH_2)_3$-3'-W_{20}-TAG-GAC-TTA-CGC-5'-$(CH_2)_6$-F [SEQ ID NO:52]
3'12F = 5'-ATC-CTG-AAT-GCG-F [SEQ ID NO:54]

Example 19: Gene Chip Assay

An ultraselective and ultrasensitive method for analyzing combinatorial DNA arrays using oligonucleotide-functionalized gold nanoparticles is described in this example. An

unusually narrow temperature range for thermal dissociation of nanoparticle-target complexes permits the discrimination of a given oligonucleotide sequence from targets with single nucleotide mismatches with extraordinary selectivity. In addition, when coupled with signal amplification method based on nanoparticle-catalyzed reduction of silver(I), the sensitivity of this nanoparticle array detection system exceeds that of the analogous, conventional fluorophore system by two orders of magnitude.

Sequence-selective DNA detection has become increasingly important as scientists unravel the genetic basis of disease and use this new information to improve medical diagnosis and treatment. Commonly used heterogeneous DNA sequence detection systems, such as Southern blots and combinatorial DNA chips, rely on the specific hybridization of surface-bound, single-strand capture oligonucleotides complementary to target DNAs. Both the specificity and sensitivity of these assays are dependent upon the dissociation properties of capture strands hybridized to perfectly-matched and mismatched targets. As described below, it has surprisingly been discovered that a single type of nanoparticles hybridized to a substrate exhibits a melting profile that is substantially sharper than both the analogous fluorophore-based system and unlabeled DNA. Moreover, the melting temperature for the nanoparticle duplex is 11 degrees higher than for the analogous fluorophore system with identical sequences. These two observations, combined with the development of a quantitative signal amplification method based upon nanoparticle catalyzed reduction of silver(I), have allowed the development of a new chip-based detection system for DNA that has single-base mismatch selectivity and a sensitivity that is two orders of magnitude more sensitive than the conventional analogous fluorescence-based assays.

Gold nanoparticles (13 nm diameter) having oligonucleotide attached to them prepared as described in Example 3 were used to indicate the presence of a particular DNA sequence hybridized to a transparent substrate in a three-component sandwich assay format (see Figure 32). In a typical experiment, a substrate was fabricated by functionalizing a float glass microscope slide (Fisher Scientific) with amine-modified probe oligonucleotides as described in Example 10. This method was used to generate slides functionalized with a